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Wild Gametophytes of *Equisetum sylvaticum*

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Though generally stated to be rare in nature, large populations of *Equisetum* gametophytes can sometimes be found on bare mud on the exposed margins of lakes and rivers in Britain. A detailed analysis of the reproductive biology and dynamics of such wild populations is given by Duckett and Duckett (1980). Apart from these littoral sites, gametophytes are occasionally seen on bare damp soil by field bryologists (Appleyard, 1981; Crundall, 1983). Elsewhere in the world *Equisetum* gametophytes have been recorded on bare mud, particularly that exposed on river banks, following the monsoon in the Indian subcontinent (Mohan-Ram & Chatterjee, 1971) or seasonal rains and the melting of winter snow in North America (Walker, 1921, 1931, 1937; Mesler & Lu, 1977; Duckett & Duckett, 1980).

The frequent occurrence of hybrid horsetails with distributions indicative of multiple origin, rather than fragmentation, and long distance dispersal of single clones (Duckett, 1979), indicates that sexual reproduction in this group of pteridophytes may not be as infrequent as normally assumed. The apparent rarity of the gametophytes is related to the production of short lived spores over a short period during each growing season (Duckett, 1970a) and narrow habitat tolerance in situations which only appear sporadically: bare mud which is neither too dry nor too wet must be available for colonization when the spores are liberated. Even after establishment the gametophytes are poor competitors with bryophytes and vascular plants and are apparently excluded from the immediate vicinity of the parent plants by allelopathic compounds (Milton & Duckett, 1985).

Detailed scrutiny of the literature on wild gametophytes reveals that the majority of observations have been made on very few species. Only those of *E. arvense* L., *E. palustre* L. and *E. fluviatile* L. have been found in Britain. I have failed to find those of *E. telmateia* Ehrh. and *E. variegatum* Schleich ex Weber and Mohr despite intensive search on many occasions of apparently suitable habitats in areas of water seepage and in wet depressions in sand dunes respectively. Attempts to establish wild gametophytes of *E. telmateia* on carefully marked sites where spores had been sown artificially were also unsuccessful (J. N. B. Milton, unpubl. data).

North American records are mainly for species in subgenus *Hippochaete* [*E. laevigatum* A.Br. (Walker, 1921, 1931), *E. scirpoides* Michx. (Walker, 1937; Feigley, 1949) and *E. hyemale* (Mesler & Lu, 1977)] plus records for *E. arvense* (Walker, 1931; Matzke, 1941; Hauke, 1967) and a single locality with *E. telmateia* (Walker, 1931). Descriptions from the Indian subcontinent are all referable to *E. ramosissimum* Desf. (Mohan-Ram & Chatterjee, 1971).

Wild gametophytes of *E. pratense* Ehrh. have never been described and the only possible record for *E. sylvaticum* L. dates from Bischoff (1853). From the description provided, plus the lack of morphological information on cultured

gametophytes at that time it is far from certain that these gametophytes can be referred to *E. sylvaticum*.

Whilst collecting bryophytes in late August 1984 on the normally inundated margin of the southern shore of Loch an Daimh, Glen Lyon, Mid West Perthshire, Scotland, Professors R. Brown, B. Lemmon (University of Louisiana) and I discovered a population of *Equisetum* gametophytes. Since *E. sylvaticum* was the only horsetail present in abundance in the vegetation surrounding Loch an Daimh, and the gametophytes differed in sexuality from those of other species of *Equisetum* previously seen in Britain, it seemed likely that the wild gametophytes were also of this species. Identification was subsequently confirmed from comparison with *E. sylvaticum* gametophytes grown in axenic culture.

This paper compares the morphology and sexual behavior of the wild gametophytes of *E. sylvaticum* with (1) gametophytes of *E. sylvaticum* in axenic culture (2) wild gametophyte populations of other species of *Equisetum*. Features of the *E. sylvaticum* habitat are compared with those of other wild gametophyte localities.

Nomenclature follows Corley and Hill (1981) for bryophytes and Clapham, Tutin, and Warburg (1962) for vascular plants.

Observations

The site.—Loch an Daimh is a reservoir constructed in the 1950s in the upper part of Glen Lyon. The elevation of the high water level is approximately 400 meters. In August 1984 the water was some 10 meters below this, having fallen steadily since the spring (D. Long, pers. comm.) during one of the driest seasons on record in the Scottish Highlands.

The vegetation bordering the south shore of the Loch is referable to the Callunetum vulgaris, Vaccineto-Callunetum and Molinieto-Callunetum associations of McVean and Radcliffe (1962), but with the addition of abundant *Equisetum sylvaticum* (20–50 stems per m^2). A few stems of *E. palustre* and *E. fluviatile* were found by the outflow stream below the dam and *E. variegatum* and *E. arvense* grew in small quantities on the sides of streamlets entering the Loch. In comparison with *E. sylvaticum* it is unlikely that these four species make a significant contribution to the spore rain.

The *Equisetum* gametophytes were restricted to a layer of sandy peat, pH 5.2, about 1 meter above the low water level along a 200 meter stretch of shore from grid reference 27/507462 to 27/502464. They were growing on both horizontal and vertical surfaces of the peat and around the remains of *Calluna* plants which predated the construction of the dam. In the immediate vicinity of the gametophytes only about 5% of the surface of the reservoir margin was covered with vegetation.

In consisting of a normally inundated reservoir margin, this site closely resembles other localities in Britain where *Equisetum* gametophytes are of regular occurrence. However, with one exception (Ladybower, Yorkshire with a value of 5.5) the pH at Loch an Daimh (pH 5.2) was significantly lower than at the other sites (6.0–7.5). The gametophytes were associated with 8 species of pha-

nerogams (highly depauperate compared with up to 80 at other sites), 23 liverworts, and 39 mosses. Most abundant were *Festuca ovina, Isolepis setacea, Pellia epiphylla, Jungermannia gracillima, Pogonatum aloides, Ditrichum heteromallum, Dicranella subulata, D. rufescens, Campylopus paradoxus, Ephemerum serratum,* var. *serratum* and *Pohlia nutans.* These species are indicative of a substrate of much lower nutrient status than that of Pennine reservoirs in Northern England (Duckett & Duckett, 1980; Duncan & Dalby, 1960). These observations on pH and associates suggest that the gametophytes of *E. sylvaticum* may have different ecological requirements from those of other horsetails.

Gametophyte morphology and sexual behavior.—The gametophytes of *E. sylvaticum* were more or less circular cushions varying from 0.5–2.0 mm in height and dark, dull green in color. They ranged in diameter from 2–9 mm (mean 4.7 mm). All the 49 individuals collected were female. None bore sporophytes.

The overall morphology of the gametophytes was identical to that of other species in the wild. Each comprised a basal cushion 10–20 cells thick bearing abundant colorless rhizoids ventrally [the morphologically similar *Fossombronia fimbriata,* also found at Loch an Daimh, has purple rhizoids (Paton, 1974)] and closely packed photosynthetic lamellae on the dorsal surface (Fig. 1). Archegonia (Fig. 2) are produced by the marginal meristem of the cushion and at maturity lie at the base of the lamellae.

Earlier attempts (Duckett, 1970, 1973) at growing spores of *E. sylvaticum* resulted solely in production of male gametophytes. However, by using Parker medium (Klekowski, 1969) on which gametophytes grow more rapidly than on the Knop and Beijerinck agars previously employed, a sexual behavior pattern similar to that in all other species of the subgenus *Equisetum* is produced. Identical results to those set out in Fig. 3 for spores from North America (Deerfield, Massachusetts) have also been obtained from British material.

The majority of the spores grow into male gametophytes which produce antheridia indefinitely. The remainder are initially female but subsequently produce antheridia. Following flooding of the latter, sporophytes (from 1–6 per gametophyte) are produced by self-fertilization.

Assuming that the wild gametophytes at Loch an Daimh derived from spores germinating in May, when collected in late August they would be approximately 125 days old. Thus their sexuality is very different from that of cultured gametophytes. Aside from the predominance of males, in culture virtually all the females have subsequently produced antheridia by this time.

It is noteworthy that Rumberg (1932), using mineral nutrient agars as culture medium, found that female gametophytes of *E. sylvaticum* subsequently produced antheridia at much the same rate as recorded here (Fig. 3), whereas those grown by Buchtien (1887) on sand and soil remained female.

The salient features of the lamellae and archegonia of *E. sylvaticum* are illustrated in Figures 1 and 2. A detailed description of the antheridia is given by Duckett (1973). Lamellae in both the cultured and wild individuals of *E. sylvaticum* are of plate type characteristic of subgenus *Equisetum* (Duckett, 1973) and as in other species, vary greatly in size and shape. However, examination of a large number reveals that apices which are obtusely lobed and flat predominate

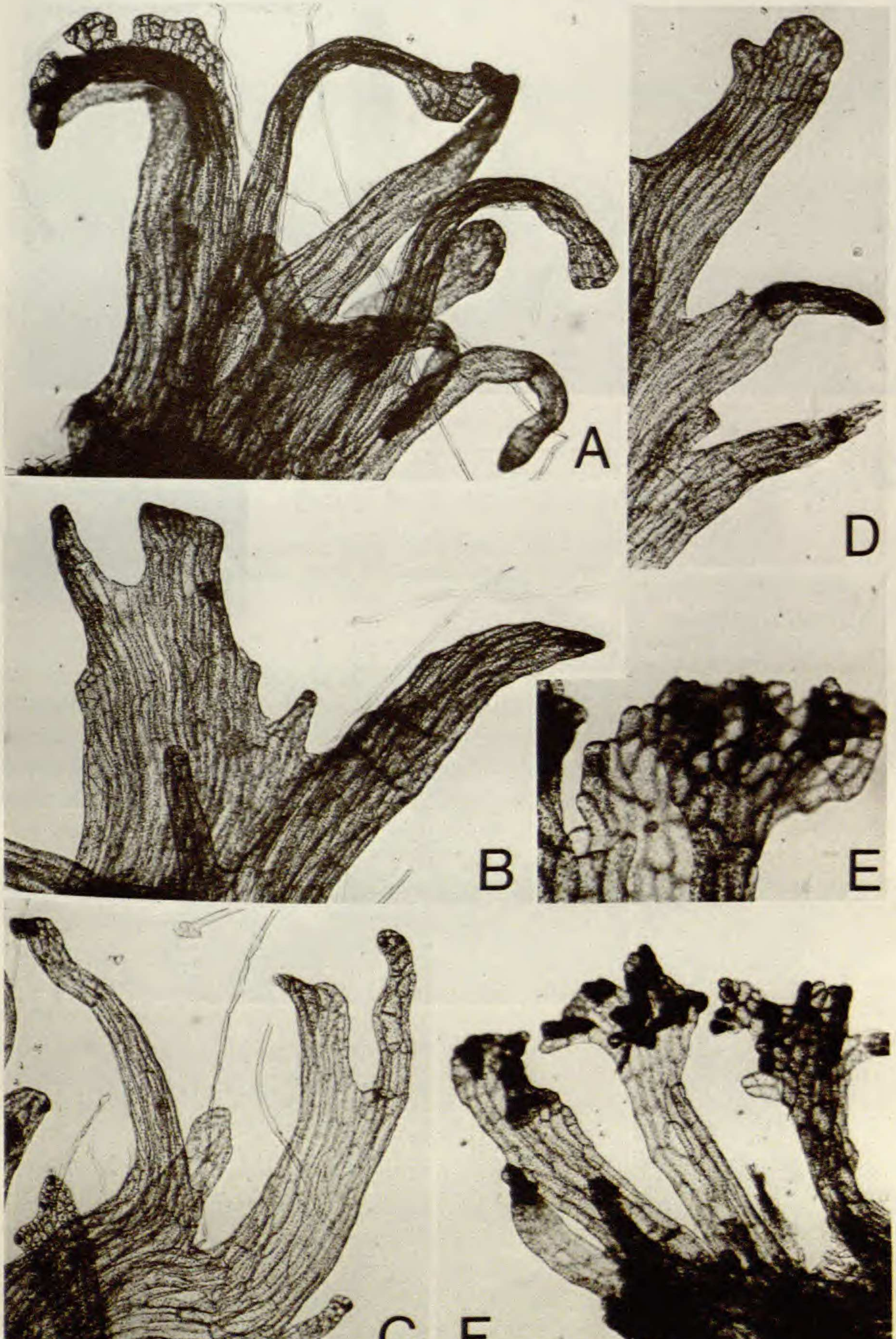

FIG. 1. Lamellae from female gametophytes of *Equisetum sylvaticum*. A–C from cultured gametophytes. D–F from wild gametophytes. All × 35 except, E, × 100.

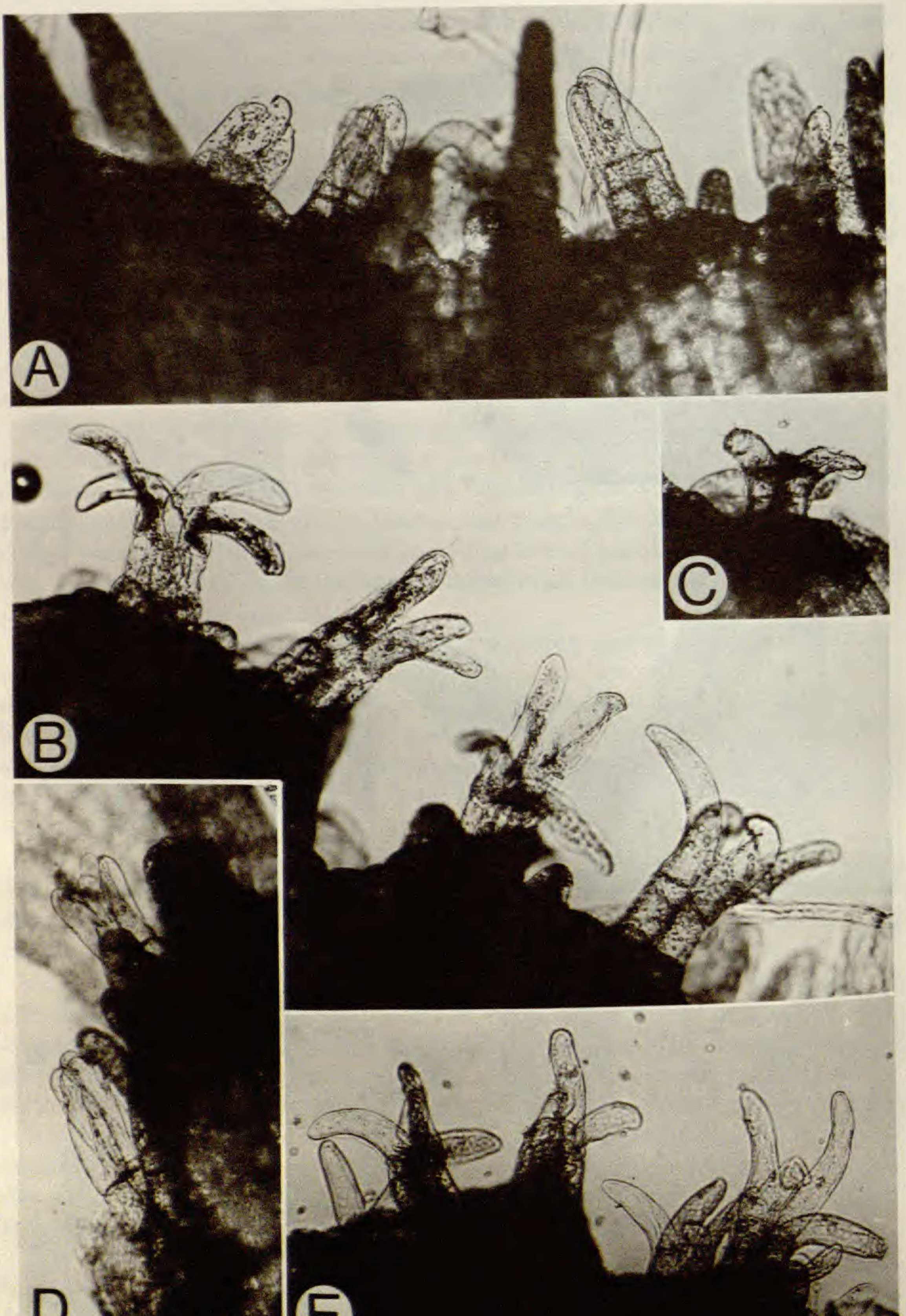

FIG. 2. Archegonia of *Equisetum sylvaticum*. A, B from cultured gametophytes. C–E from wild gametophytes. All × 100.

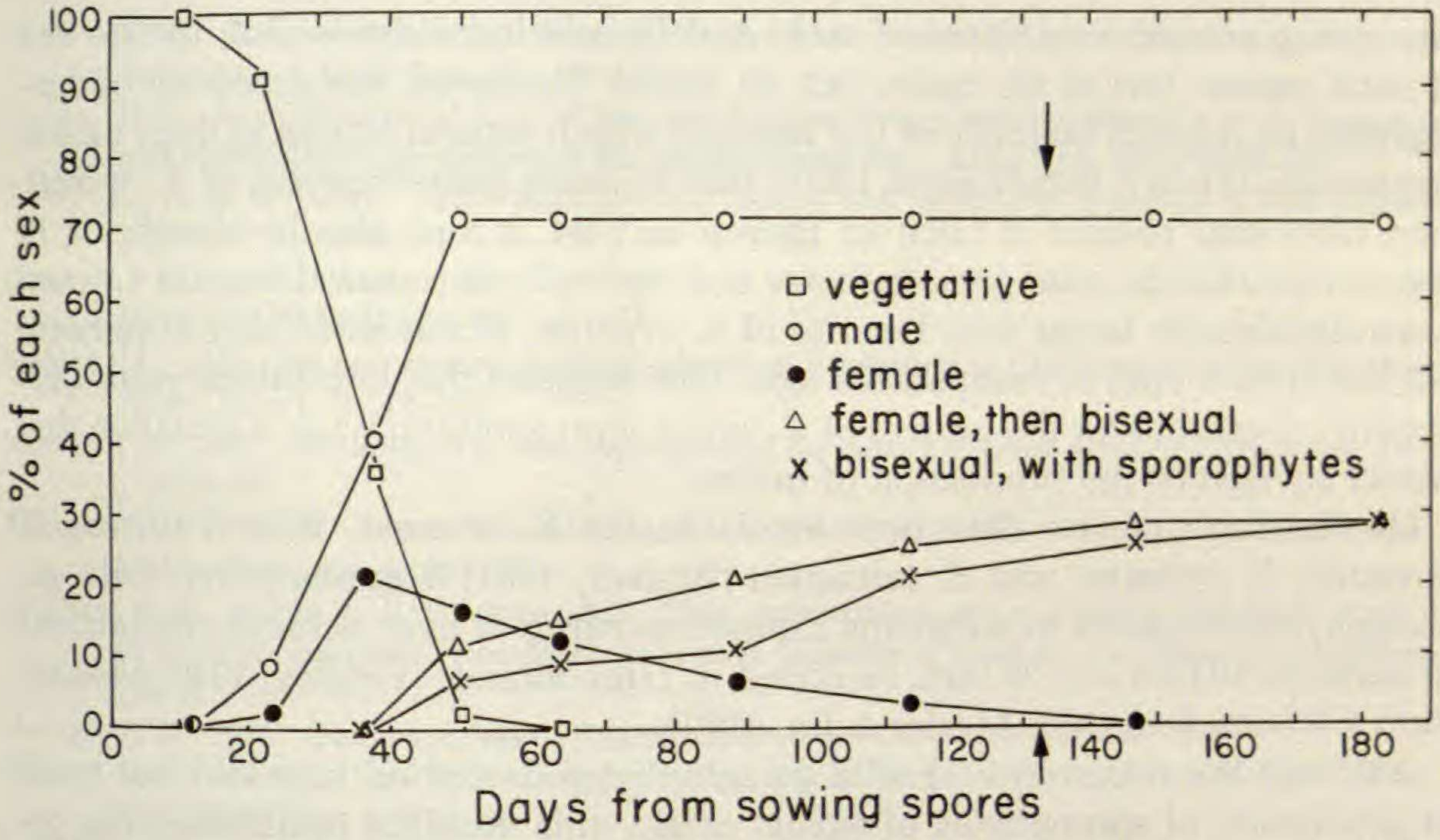

FIG. 3. Sexual behavior of gametophytes of *Equisetum sylvaticum* grown in single spore cultures on Parker medium (Klekowski, 1969). 300 spores, collected from Deerfield, Massachusetts, were cultured as set out in Duckett (1970). Arrows indicate the approximate age of the wild gametophytes.

(cf. the funnel-shaped apices of *E. arvense*). The apical cells are usually short whereas those in *E. telmateia* and *E. fluviatile* are elongate. In gross morphology the lamellae of *E. sylvaticum* are closest to those of *E. palustre*. The latter species however possesses apical cells that are usually less than 30–40 μm wide whereas those of *E. sylvaticum* mostly fall into the size range 30–60 μm.

In common with other members of the genus, archegonia of *E. sylvaticum* possess protruding necks composed of three tiers, each of four cells. At maturity the cells of the terminal tier elongate considerably (up to six times their original length), separate from each other, and bend away from the neck canal. This maturational divergence appears to be more variable in *E. sylvaticum* than in other species of *Equisetum*. Prior to divergence the terminal neck cells are slightly twisted both in cultured (Fig. 2A) and wild (Fig. 2D) specimens. The subterminal neck cells of *E. sylvaticum* are much shorter than those of *E. fluviatile* and *E. arvense* (Duckett & Duckett, 1980).

DISCUSSION

On the basis of morphology, sexuality, and availability of spores in the adjacent vegetation, there seems to be little doubt that the wild gametophytes from the shore of Loch an Daimh are referable to *E. sylvaticum*. Although the gross morphology of these wild gametophytes is the same as that of other species in nature, they differed in two major respects; sexuality and the absence of sporophytes.

Wild gametophyte populations of *E. arvense*, *E. palustre*, and *E. fluviatile* in

late spring comprise mixtures of male and female individuals. But, by the end of each season few or no males can be found. However, the presence of sporophytes on the vast majority of the females which remain attests to their earlier occurrence. Thus it would seem likely that no male gametophytes of *E. sylvaticum* were ever present at Loch an Daimh in 1984. It may also be significant in this context that the wild gametophytes of *E. sylvaticum* (mean diameter 4.7 mm) were significantly larger than females of *E. arvense*, *E. fluviatile*, and *E. palustre* (all less than 3 mm) of comparable age. This suggests that conditions were particularly favorable for the growth of *E. sylvaticum* gametophytes, a situation that would act against the production of males.

On the basis of size data now available for *E. arvense*, *E. sylvaticum*, *E. fluviatile*, *E. palustre*, and *E. telmateia* (Walker, 1931) it appears that wild gametophytes of species in subgenus *Equisetum* rarely if ever achieve dimensions of between 10 mm and 35 mm, recorded in *Hippochaete* (Feigley, 1949; Mohan-Ram & Chatterjee, 1971; Mesler & Lu, 1977).

Although the occurrence of wild gametophytes described here did not result in production of sporophytes of sexual origin, this incident establishes the potential for sexual reproduction in *E. sylvaticum*.

At some similar site, but where *E. pratense* grows alongside *E. sylvaticum* one may readily envisage the origin of *E.* ×*mildeanum* Rothm., the hybrid involving the two species in subsection *Subvernalia* (Duckett, 1979). When grown axenically under identical culture regimes each species of *Equisetum* has a distinct pattern of sexual behavior (Duckett, 1970, 1972). Specific differences in the proportions of males and females in culture are paralleled in mixed species gametophyte populations found in the wild. Two hybrid horsetails, *E. arvense* × *E. fluviatile* and *E. fluviatile* × *E. palustre* have already been found to arise from such situations (Duckett & Duckett, 1980). Under the culture regime used in this investigation 27% of the spores of *E. sylvaticum* produced female gametophytes but the corresponding figure for *E. pratense* was only 8% (Duckett, unpubl. data). A similar difference in sexuality in the wild would clearly favor the production of hybrid sporophytes.

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